

Abstract

Even though prophylactic vaccine against human papillomavirus (HPV) is currently licensed, infections by the virus continue to be the major health problem mainly in developing countries. Considerable effort is being devoted to preparation of therapeutic vaccine and to decrease of the production costs of current vaccine. Viral proteins such as the E7 oncoprotein and the L2 capsid protein from HPV type 16 are promising targets for the development of the experimental anti-HPV vaccine.

The aim of our work was optimization of expression of mutagenized E7 oncoprotein (E7ggg) fused to the C-terminus of *Tobacco mosaic virus* (TMV) coat protein (CP) or *Potato virus X* (PVX) CP in viral vectors derived from these plant viruses. The impact of linkers connecting CP and E7ggg fusion partners on expression and stability of fusion proteins was examined. The fusion proteins were first expressed in *Escherichia coli* (*E. coli*) MC1061 to assess the characteristics of the recombinant protein prior to their transient expression in both non-transgenic or transgenic *Nicotiana benthamiana* (*N. benthamiana*). We have obtained the high level expression in *E. coli*, but most of the expressed proteins based on TMV CP remained in insoluble inclusion bodies. To increase the ratio of soluble protein various molecular chaperones (Trigger factor (TF), DnaK-DnaJ-GrpE, GroEL-GroES) were used. The L2₁₀₈₋₁₂₀ epitope fused to the C-terminus of PVX CP was successfully expressed in transgenic *N. benthamiana* carrying gene for movement protein (MP) from TMV. Fusion of E7ggg or L2₁₀₈₋₁₂₀ to the C-terminus of PVX CP is the first report of the C-terminal protein/epitope fusion with PVX CP *in planta*.

Other topic solved in our work was the characterization of some properties of expressed fusion proteins like solubility, stability, immunological reactivity and ability to form virus-like particles (VLP).

Biotechnological use of transient expression of pharmaceutical proteins in plants using viral vectors derived from plant viruses seems to be a promising nascent technology for production of experimental vaccines and pharmaceutical proteins in plants.

Key words: human papillomovirus (HPV), HPV E7 oncoprotein, HPV L2₁₀₈₋₁₂₀ epitope, bacterial expression, transient expression in plants